Materials and Methods
MATERIALS AND METHODS

SELECTION OF THE PLANT

Nature has continuously provided mankind with a broad and structurally diverse array of pharmacologically active compounds that have proved to be indispensable for the cure of deadly diseases or as lead structures for novel pharmaceuticals.

Natural products have been recognized as an important tool in the drug discovery process throughout this century (Strohl, 2000; Agosto, 1997). Of the 20 best-selling non-protein drugs in 1999, nine were natural products or products derived from them, with combined annual sales of up to US $16 billion. Presently, over 100 natural product-derived pharmaceuticals are used in medicine (Harvey, 2000; Verpoorte, 1998).

According to the WHO, about three-quarters of the world population rely upon traditional remedies (mainly herbs) for their health care. In fact, herbs/plants are the oldest friends of mankind. They not only provide food and shelter but also serve the humanity to cure different ailments. The herbal medicine also sometime called as, traditional or natural medicine existed in one way or another in different cultures/civilizations, such as Egyptians, Western, Chinese, Kampo (Japan) and Greco-Arab or Unani/Tibb (South Asia).

Historians from all around the world have produced evidence to show that apparently all primitive people used herbs-often in a sophisticated way. Based on their experience they are using the plants to cure various ailments.
Literature survey indicates that several plants were identified in Siddha literature as potential diuretics which are used by Siddha practitioners to treat Edema, Hydronephrosis and Urinary calculi. One of the Siddha pharmaceutical industries in Namakkal District, Tamilnadu has been promoting a Siddha proprietary drug in the form of capsule named Stonsil to treat Urinary Calculi and Hydronephrosis. In the above preparation the following two plants are mainly used. It was of interest to us to study the plants used by them as diuretic to validate them. The two potential plants selected for our study are:

1. *Aerva lanata* (Linn.) Juss.

2. *Tribulus terrestris* Linn.

The above plants are cosmopolitan and available plenty in the wild.

**Collection and authentication of plant**

The plant of *Aerva lanata* and *Tribulus terrestris* were collected from the plains of the Namakkal District, in the month of June 2002. The collected plants were identified and authenticated by a Botanist Prof. Dr. K. Sigamani, Head, Department of Botany, Kandasamy Kandar College, Velur, Namakkal District, Tamilnadu, India.

**PHARMACOGNOSTICAL STUDIES**

**Microscopical studies**

The plant specimens for the proposed study were collected from the Namakkal District. Care was taken to select healthy plants and for normal organs. The required samples of different origins were cut and removed from the plant and fixed in FAA (Formalin- 5 ml + Acetic acid- 5 ml+70% ethyl alcohol-90 ml). After 24 hrs of fixing, the specimens were dehydrated with graded series of tertiary-Butyl alcohol as per the
schedule given by Sass, 1940. infiltration of the specimens was carried by gradual addition of paraffin wax (mp. 58-60° C) until TBA solution attained supersaturation. The specimens were cast into paraffin blocks.

Sectioning

The paraffin embedded specimens were section with the help of Rotary Microtome. The thickness of the sections was 10-12 μm. Dewaxing of the sections was by customary procedure (Johansen, 1940). The sections were stained with Toluidine blue as per the method published by O’Brien et al. (1964). Since Toluidine blue is a polychromatic stain, the staining results were remarkably good; and some cytochemical reactions were also obtained. The dye rendered pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, violet to the mucilage, blue to the protein bodies etc. wherever necessary sections were also stained with safranin and fast-green and IKI (for starch).

For studying the stomatal morphology, venation pattern and trichome distribution, paradermal sections (sections taken parallel to the surface of leaf) as well as clearing of leaf with 5% sodium hydroxide or epidermal peeling by partial maceration employing Jeffrey’s maceration fluid (Sass, 1940) were prepared. Glycerine mounted temporary preparations were made for macerated/cleared materials. Powdered materials of different parts were cleared with NaOH and mounted in glycerine medium after staining. Different cell component were studied and measured.

Photomicrographs

Microscopic descriptions of tissues are supplemented with micrographs wherever necessary. Photographs of different magnifications were taken with Nikon Lab phot 2 Microscopic unit. For normal
observations bright field was used. For the study of crystals, starch grains and lignified cells, polarized light was employed. Since these structures have birefringent property, under polarized light they appear bright against dark background. Magnifications of the figures are indicated by the scale-bars. Descriptive terms of the anatomical features are as given in the standard anatomy books (Esau, 1964).

**Extractive values** (Trease and Evans, 1988)

**i) Alcohol soluble extractive value**

5 gms of shade dried coarse powdered aerial parts of *Aerva lanata* (Linn) Juss. and *Tribulus terrestris* Linn. were macerated with 100 ml of alcohol (90% v/v) in a closed flask for 24 Hrs, shaking frequently during 6 Hrs and allowed to stand for 18 Hrs. Filtered immediately, taking precautions against loss of alcohol. 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish. Dried at 105°C and weighed. The percentage of alcohol soluble extractive with reference to air dried drug was calculated.

**ii) Water soluble extractive value**

5 gms of shade dried coarse powdered aerial parts of *Aerva lanata* (Linn) Juss. and *Tribulus terrestris* Linn. were macerated with 100 ml of chloroform water (1 ml of chloroform in 100 ml of water) in a closed flask for 24 Hrs, shaking frequently during 6 Hrs and allowing to stand for 18 Hrs. Filtered rapidly, taking precautions against loss of chloroform water. 25 ml of the filtrate was evaporated to dryness in a tared flat bottomed shallow dish. Dried at 105°C and weighed. The percentage of water soluble extracts with reference to air dried drug was calculated.

**Extraction of plant material** (Kokate, 1994)

The collected, cleaned and powdered aerial parts of *Aerva lanata* and *Tribulus terrestris* were used for the extraction purposes. 1 Kg of
powdered aerial part was evenly packed in the soxhlet apparatus. It was then extracted with various solvents from nonpolar to polar such as petroleum ether, chloroform, acetone, ethyl acetate ethanol and aqueous successfully. The solvents used were purified before use. The extraction was carried out with various solvents by hot continuous extraction for 72 Hrs. After each solvent extraction the extracts were filtered while hot through Whatmann filter paper to remove any impurities if present.

Materials required

Shade dried aerial parts of *Aerva lanata* and *Tribulus terrestris*, petroleum ether LR, Chloroform LR, Acetone LR, Ethyl acetate LR, Ethanol and 0.25% Chloroform Water.

a) Petroleum ether extracts of *Aerva lanata* and *Tribulus terrestris*

About 1 kg of dry powders was extracted first with 2-3 litres of petroleum ether 60°- 80°C by continuous hot percolation using soxhlet apparatus. After completion of extraction the petroleum ether extracts were filtered and concentrated to dry mass by vacuum distillation. The extracts were then stored in a desiccator.

b) Chloroform extracts of *Aerva lanata* and *Tribulus terrestris*

The marc left after pet ether extract were dried and subsequently extracted with 2-3 litres of chloroform (59.1° - 61.5°C) by continuous hot percolation using soxhlet apparatus. After completion of extraction, it was filtered and the solvent was removed by distillation under reduced pressure. The extracts were then stored in a desiccator.

c) Acetone extracts of *Aerva lanata* and *Tribulus terrestris*

The marc left after chloroform extract was dried and subsequently extracted with 2-3 litres of acetone (55.5° - 56.5°C) by continuous hot
percolation using soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extracts were then stored in a desiccator.

d) Ethyl acetate extracts of *Aerva lanata* and *Tribulus terrestris*

The marc left after acetone extract was dried and subsequently extracted with 2-3 litres of acetone (55.5° - 56.5°C) by continuous hot percolation using soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extracts were then stored in a desiccator.

e) Ethanolic extracts of *Aerva lanata* and *Tribulus terrestris*

The marc left after ethyl acetate extract was dried and subsequently extracted with 2-3 litres of Ethanol 95% by continuous hot percolation using soxhlet apparatus. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extracts were then stored in a desiccator.

f) Aqueous extracts of *Aerva lanata* and *Tribulus terrestris*

The marc left after ethanolic extraction was taken and finally macerated the marc with 2-3 litres of chloroform water (0.25% CHCl₃) in a narrow mouthed bottle for three days. After completion of extraction it was filtered and the solvent was removed by distillation under reduced pressure. The extracts were then stored in a desiccator.

From the weight of the each extractive residue, the extractive values were calculated in percentage. All the above extracts were used for identification of constituents by phytochemical tests and for the pharmacological studies.
IDENTIFICATION OF PLANT CONSTITUENTS BY PHYTOCHEMICAL TESTS (IP, 1996; Kokate, 1994; Harbone, 1973)

The various extracts of the aerial parts of *Aerva lanata* and *Tribulus terrestris* were subjected to chemical tests for identification of its active constituents.

Tests for alkaloids

A small portion of the solvent free chloroform, ethanolic and aqueous extracts were stirred separately with a few drops of dil. hydrochloric acid and filtered. The filtrate may be tested carefully with various alkaloidal reagents such as

(a) Mayer's reagent - yellow precipitate
(b) Dragendorff's reagent - orange brown precipitate
(c) Hager's reagent - yellow precipitate
(d) Wagner's reagent - Reddish brown precipitate

Test for carbohydrates and glycosides

A small quantity of extracts were dissolved separately in 5 ml of distilled water and filtered. The filtrate was subjected to Molisch's test to detect the presence of carbohydrates.

(a) Molisch's test

Filtrate was treated with 2-3 drops of 1% alcoholic α - naphthol solution and 2 ml of concentrated sulphuric acid was added along the sides of the test tube. Appearance of brown ring at the junction of two liquids shows the presence of carbohydrates.

Another portion of the extract was hydrolysed with hydrochloric acid for few hours on a water bath and the hydrolysate was subjected to
Legal's and Borntrager's tests to detect the presence of different glycosides.

(b) Legal's test

To the hydrolysate, 1 ml Pyridine and few drops of sodium nitroprusside solution were added and then it was made alkaline with sodium hydroxide solution. Appearance of pink to red colour shows the presence of glycosides and aglycones.

(c) Borntrager's test

Hydrolysate was treated with chloroform and the chloroform layer was separated. To this equal quantity of dilute ammonia solution was added. Ammonical layer acquires rose pink colour shows the presence of glycosides.

Test for phytosterol

Libermann Burchard test

One gram of the extract was dissolved in few drops of dry acetic acid, 3 ml of acetic anhydride was added followed by few drops of conc. sulphuric acid. Appearance of bluish green colour shows the presence of phyto sterol.

Test for fixed oils and fats

(a) Small quantities of the various extracts were separately pressed between two filter papers. Appearance of Oil stain on the paper indicates the presence of fixed oil.

(b) Few drops of 0.5N alcoholic potassium hydroxide were added to a small quantity of various extracts along with a drop of phenolphthalein. The mixture was heated on water bath for 1-2
hours. Formation of soap or partial neutralisation of alkali indicates the presence of fixed oil and fats.

**Test for saponins**

The extracts were diluted with 20ml of distilled water and it was agitated on a graduated cylinder for 15 mins. the formation of 1 cm layer of foam shows the presence of saponins.

**Test for tannins and phenolic compounds**

Small quantities of various extracts were taken separately in water and test for the presence of Phenolic compounds and Tannins with

(i) Dilute ferric chloride solution (5%) - Violet colour
(ii) 1% solution of gelatin containing 10% Nacl - White precipitate
(iii) 10% lead acetate solution - White precipitate.

**Test for proteins and free amino acids**

Small quantities of various extracts were dissolved in a few ml of water and treated with

(i) **Millons's reagent** - Appearance of red colour shows the presence of proteins and free amino acids.

(ii) **Ninhydrin reagent** - Appearance of purple colour shows the presence of proteins and free amino acids.

(iii) **Biuret Test** - equal volume of 5% sodium hydroxide solution and 1% copper sulphate solution were added. Appearance of pink or purple colour shows the presence of proteins and free amino acids.

**Test for gums and mucilages**

About 10 ml of various extracts were added separately to 25 ml of absolute alcohol with constant stirring and filtered. The precipitate was
dried in air and examine for its swelling properties and for the presence of carbohydrates.

**Test for flavonoids**

a) **With aqueous sodium hydroxide solution** - blue to violet colour (anthocyanins), yellow colour (Flavones), Yellow to orange (flavonones).

b) **With concentrated sulphuric acid** - Yellowish orange colour (Anthocyanins), yellow to orange colour (Flavones), orange to crimson (flavonones).

c) **Shinoda's test** - The extract is dissolved in alcohol, with piece of magnesium followed by conc. hydrochloric acid drop wise are added and heated. Appearance of majenta colour shows the presence of flavonoids.

**Test for lignin**

With alcoholic solution of phloroglucinol and Hydrochloric acid. Appearance of red colour shows the presence of lignin.

**PHARMACOLOGICAL SCREENING**

**Acute toxicity studies** (Ghosh, 1984)

Toxicity studies involves a test in which single dose of the drug given used in each animal on one occasion only for the determination of LD$_{50}$ or median lethal dose, ie. the dose which kills the 50% animals of a particular species. LD$_{50}$ value was determined in 24 hrs.

The acute toxicity test aims at establishing the therapeutic index, ie the ratio between the pharmacologically effective dose and lethal dose on the same strain and species (LD$_{50}$/ED$_{50}$). The greater the index the safer
the compound and vice versa. Because of the species variation several species of animals are used to determine the LD$_{50}$. In practice, however, it is not necessary to establish LD$_{50}$ figures for a whole range of species. It is considered sufficiently adequate if LD$_{50}$ with confidence limits is established on one common laboratory species, such as mice or rats by the standard method.

For calculating LD$_{50}$, the least tolerated (smallest) dose (100% mortality) and most tolerated (highest) dose (0% mortality) were found out by hit and trial method. Once these two doses are determined, at least 5 doses in between the least tolerated and most tolerated doses were selected and the mortality due to these doses was observed. Generally mice are used for this purpose and each dose group should consist of 10 animals. One can determine the LD$_{50}$ value by different routes of administration. The LD$_{50}$ value of the new drug is determined by oral as well as by one of the parenteral routes (IP, IV or IM) of administration (Kulkarni, 1993).

**Materials required**

**Animals** : Swiss albino mice of either sex (20-25 grams)

**Drugs** : Ethanolic extract of aerial parts of *Aerva lanata* and *Tribulus terrestris*.

**Methodology**

The overnight fasted Swiss mice were weighed and divided into twelve groups of ten each (20-25 grams). Group 1 to 6 received separately the various doses of aerial part extracts of *Aerva lanata* and *Tribulus terrestris* separately through (700mg/Kg, 1400mg/Kg, 2100mg/Kg, 2800mg/Kg, 3500mg/Kg and 4200mg/Kg) orally through gastric gavage.
tube. After the administration of the extract, the animals were observed continuously for the first two hours for death due to acute toxicity. The results of LD$_{50}$ study done in mice using Karbers method are shown in the table. The correction factor is applied from 0 to 100 percent mortality group.

The percent mortality values were converted to probit values by reading the corresponding probit units from the probit table. The probit values were plotted against log doses. From this, LD$_{50}$ value was read as the dose that corresponds to probit 5.

**DIURETIC ACTIVITY**

**Materials required**

**Animals** : Wister rats of either sex (125-150 gm)

**Drugs** : Ethanolic extract of aerial parts of *Aerva lanata* and *Tribulus terrestris*.

**Standard drug** : Furosemide

**Methodology**

The diuretic effects of ethanolic extracts of *Aerva lanata* and *Tribulus terrestris* Linn. were evaluated in normal rats. The method of Lipschitz *et al.* (1943) as modified by Kau *et al.* (1984) was adopted for this test. The animals, fasted and deprived of water for 18 h prior to the experiment, were divided into six groups of six rats each. Then, all animals were given an oral loading of normal saline (5% bw). The first group of animals, serving as control, received normal saline (10 ml/kg, p.o.); the second group received furosemide (1 mg/kg, p.o.) (Jouad *et al.*, 2001) as a standard. The third, fourth, fifth and sixth groups received the test extracts (ethanolic extracts of *Aerva lanata* and *Tribulus terrestris*.
Linn. and) at doses of 250 and 500 mg/kg, respectively. Immediately after dosing, the animals were separately placed in metabolic cages with provision for urine collection in graduated measuring cylinders. The experimental protocol adopted has two phases: a control phase of 1 h and an experimental phase of 4 h. Three urine and blood samples were taken within the experimental phase at the start, 2 and 4 h of treatment. All experiments performed while animals were deprived of food and water.

ANTI-INFLAMMATORY ACTIVITY

Materials required

Animals : Wister rats of either sex (125-150 gm)
Drugs : Ethanolic extract of aerial parts of *Aerva lanata* and *Tribulus terrestris*.
Standard drug : Indomethacin
Equipment : Plethysmograph.

Methodology

Carrageenan induced acute paw oedema method (Winter et al, 1968) was employed for the assessment of anti-inflammatory activity. Wister rats of either sex (125-150 gms) were divided into four groups of six each.

First group (negative control) received 1 ml of normal saline, second group (positive control) received 10 mg/kg p.o., Indomethacin, third and fourth group received ethanolic extract (250 mg/kg, p.o.,) of *Aerva lanata* and *Tribulus terrestris*, respectively. After 1 h, the rats were challenged with subcutaneous injection of 0.1 ml of 1 % w/v solution of carrageenan (Sigma chemical co, St. Louis MO, USA) into the plantar side of the left hind paw. The paw was marked with ink at the level of lateral malleolus and immersed in mercury up to the mark. The
plethysmograph apparatus used for the measurement of rat paw volume was that of Singh and Ghosh (1968). The paw volume was measured immediately after injection (0 h) and followed by every hour till the 3 h after injection of carrageenan to each group. The difference between the initial and subsequent reading gave the actual edema volume.

Percent inhibition of inflammation was calculated using the formula, \( % \text{ inhibition} = 100 \left( 1 - \frac{V_t}{V_c} \right) \), where 'Vc' represents edema volume in control and 'Vt' edema volume in group treated with test extracts.

**ANTIMICROBIAL ACTIVITY**

The antibacterial activity of the chloroform and ethanolic extracts were studied systematically against four different strains of bacteria by agar diffusion method in particular cup-plate method (BP, 1993; IP, 1985). In this method, cups of standard diameter are made in the nutrient agar medium containing standard bacterial inoculum. The text extracts were introduced into these dishes and the diameters of the zone of inhibition in mm were measured after the incubation.

The text extracts were evaluated for its antibacterial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus pyogenes* and *Escherichia coli* by the agar diffusion method.

The test organisms were subcultured using nutrient agar medium. The tubes containing sterilised medium were inoculated with respective bacterial strain. After incubation at 37±1°C for 24 Hrs, they were stored in refrigerator. Thus stock cultures were maintained. Bacterial inoculum was prepared by transforming a loopful of stock culture to nutrient broth.
The extracts were dissolved with 0.5% Carboxy Methyl Cellulose solution to make 10 mg/ml solution. A reference standard was also prepared by dissolving ampicillin in water for injection (10 mg/ml). The medium was sterilized by autoclaving at 120° (15 lb/in²). About 30 ml of molten nutrient agar medium inoculated with the respective strain of bacteria (6 ml of inoculum to 300 ml of nutrient agar medium) was transferred aseptically into each sterilized petri plate (10 cm diameter). The plates were left at room temperature to allow solidification. In each plate 3 wells of 6 mm diameter were made with a sterile borer. Accurately 0.2 ml of the test solution was added to the cups aseptically and labeled accordingly. After incubation of the plates at 37±1° for 24 h, the diameter of the zone of inhibition surrounding each of the wells was noted. Simultaneously control was maintained employing 0.2 ml of CMC sodium solution (0.5%) to observe the solvent effect.

TOXICOLOGICAL STUDIES

Materials required

Animal : Wistar rats of either sex (125-150 gm)

Drugs : Alcoholic extract (250, 500, 750 and 1000 mg/Kg, p.o.) of *Aerva lanata* and *Tribulus terrestris*.

Methodology

Nine groups of rats were used for the toxicological studies of *Aerva lanata* and *Tribulus terrestris* each consists of 6 animals. Group I served as control, treated with normal saline 2ml/kg. the second, third, fourth and fifth group received 250, 500, 750 and 1000 mg/kg, p.o., of ethanolic extract of *Aerva lanata*, respectively. The sixth, seventh, eighth and ninth group of animals received 250, 500, 750 and 1000 mg/kg, p.o., of ethanolic extract of *Tribulus terrestris*, respectively. The animals were
received the extracts once a day for 28 days. The animals were observed for signs and symptoms, behaviour alteration, food and water intake and the body weight changes. Blood samples were collected after the last dose of ethanolic extract. The haematological parameters such as Hb, RBC, WBC, ESR and PCV were determined by using the routine methods (Dacie, 1958) in the above group of animals.

In addition blood glucose, cholesterol, ALT (Reitman and Frankel, 1957), AST (Armilage and Berry, 1985), ALP (Kind and King, 1954), total bilirubin (Burtis and Ashwood, 1996), total protein (Reinhold 1953; Henry et al 1974) urea (Natelson, 1957), creatinine (Hendry, 1974) and GGTP (Szasz, 1969) were estimated as reported earlier. Same time the liver and kidney of the animals were removed and subjected to examination and later weighed.

Since the liver and kidney are the organs of metabolism and excretion, potentially toxic agents are likely to affect them. A small portion of liver and kidney was cut from the animals from each group and preserved in neutral buffered formalin and was processed for paraffin embedding, following the standard microtechnique (Galigher and Kozloff, 1971). 5μ sections of the livers stained with alum haemotoxylin and eosin and studied for degenerative and necrotic changes.

**Statistical analysis**

All values were expressed as mean±SEM. The data were statistically analyzed using one way ANOVA followed by Newman Keul’s multiple range test and differences below P<0.05 are considered as significant.
ISOLATION AND CHARACTERISATION

Isolation of active constituents

Column chromatography

Column chromatography (Beckett stenlake, 1986; Furniss et al., 1982) is widely used method to isolate the active constituents, in its pure form, from the crude extract or fractions. The main principle involved in the column chromatography is adsorption at the solid liquid interface.

For successful separation, the compounds of a mixture must show different degrees of affinity for the solid support (or adsorbent) and the interaction between adsorbent and component must be reversible.

As the adsorbent is washed with fresh solvent, the various components will move down the column until, ultimately, they are arranged in order of their affinity for the adsorbent. Those with least affinity move down the column at a faster and are eluted first and those with greater affinity for the adsorbent are eluted faster.

Materials

1. Glass column of size 60cm x 2 cm
2. Silica gel for column chromatography 60-120 mesh (Qualigens) as the adsorbent.

Preparation of the column

The silica gel 60-120 mesh was made into slurry with petroleum ether (First eluent). The bottom of the column was plugged with little glass wool and then clean sand bed was placed over the glass wool. The sand bed serves to give a flat base to the column of the adsorbent. Then the slurry was poured into the column, which was filled with solvent.
After 2/3rd of the column was filled with the slurry, it was set aside for 10 minutes and allowed to settle.

After the adsorbent has settled, a filter paper disc and sand bed was placed over the adsorbent, in order to avoid the disturbance of the top layer of the adsorbent as fresh mobile phase was added to the column in the initial stages of development.

**Development of chromatography**

The crude ethanolic extract of *Aerva lanata* and *Tribulus terrestris* were subjected to column chromatography over silica gel 60-120 mesh. The column was eluted with solvents of increasing polarity. The solvents used were petroleum ether, chloroform, acetone, ethyl acetate, alcohol and aqueous.

The fractions were collected in 10ml each and allowed to evaporate to get the residue. Each fraction was tested for the presence of various constituents by thin layer chromatography for the number and type of constituents.

Brown residue was obtained from crude ethanolic extract of *Aerva lanata* column with Ethylacetate: Alcohol (20:80) and yellowish residue was obtained from the crude extract of *Tribulus terrestris* column with Ethylacetae:Alcohol (60:40). Purity of residue was checked by TLC with various solvent systems.

Yield of the compound was found to be 0.08652 gm and 0.07562 gm for the ethanolic extract of *Aerva lanata* and *Tribulus terrestris*, respectively. The compounds were undergone spectral studies of the details were shown in results and discussion.
Table 4. The ratios of the eluent used for column chromatography.

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<th>S.No</th>
<th>Eluent</th>
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<tr>
<td>1</td>
<td>Petroleum Ether</td>
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<tr>
<td>36</td>
<td>Ethylacetate: Alcohol</td>
<td>50:50</td>
</tr>
<tr>
<td>37</td>
<td>Ethylacetate: Alcohol</td>
<td>40:60</td>
</tr>
<tr>
<td>38</td>
<td>Ethylacetate: Alcohol</td>
<td>30:70</td>
</tr>
<tr>
<td>39</td>
<td>Ethylacetate: Alcohol</td>
<td>20:80</td>
</tr>
<tr>
<td>40</td>
<td>Ethylacetate: Alcohol</td>
<td>10:90</td>
</tr>
<tr>
<td>41</td>
<td>Alcohol</td>
<td>100</td>
</tr>
</tbody>
</table>
CHARACTERISATION OF THE ISOLATED PLANT CONSTITUENTS
(John Dyer 1978; Silverstein 1982; Willard et al., 1983)

Instruments used

1. Perkin-Elmer Vector 22 model FT-IR spectrophotometer (Nujol).
2. Bruker DPX-200 FT-NMR spectrophotometer (German make)
   (Proton and Carbon)
3. GCMS – Shimadzu QP 5000 (Japan make).

Description of the compounds

The description of the compounds isolated from the crude ethanolic extract of *Aerva lanata* and *Tribulus terrestris* was tabulated in Table 5. Elution of the compound from crude alcohol extract column with Ethylacetate: alcohol (20:80).
Table 5. Description of the isolated compounds.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Description</th>
<th>Aerva lanata</th>
<th>Tribulus terrestris</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Colour</td>
<td>Brown amorphous waxy solid</td>
<td>Yellowish amorphous waxy solid</td>
</tr>
<tr>
<td>3</td>
<td>Solubility</td>
<td>Soluble in DMSO, Chloroform</td>
<td>DMSO</td>
</tr>
<tr>
<td>4</td>
<td>Melting point</td>
<td>40-42°C.</td>
<td>54-56°C</td>
</tr>
<tr>
<td>5</td>
<td>Rf value</td>
<td>0.82 (Benzene: Methanol 90:10)</td>
<td>0.75 (Acetone: ethyl acetate 70:30)</td>
</tr>
</tbody>
</table>